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Ectomycorrhiza-hydrogel additive enhanced growth of Norway spruce seedlings in a nutrient-poor peat substrate

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Abstract: Seedling quality is an important input affecting the outplanted seedling performance. Morphological attributes and association with symbiotic ectomycorrhizal (ECM) fungi belong to influential traits determining seedling quality. In this study, the effect of pre-sowing applied commercial additives Ectovit (containing hydrogel and ECM fungi), Mycorrhizaroots (ECM fungi and nutrients) and Vetozen (natural mineral fertilizer) on the growth and ectomycorrhiza formation of Norway spruce seedlings grown in nutrient-poor pure peat in an open nursery site was assessed. Two-year-old bareroot seedlings were transplanted into containers. No significant growth differences were detected between treatments for 1 + 0 seedlings but the values of growth parameters (root collar diameter; stem height; shoot, root and total dry weight) of Ectovit-inoculated seedlings were significantly higher than those in the other treatments including the control after the second (2 + 0 seedlings) and the third growing season (2 + 1). Root-to-shoot dry weight ratio and number of root tips were distinctly higher after the third year compared to the previous two years but they were not significantly different between treatments. Mycorrhizaroots and Vetozen did not have any significant effect on seedling development. Although the occurrence of treatment-specific ECM morphotype was detected in Ectovit-inoculated seedlings, the high total ECM colonization of roots in all treatments including the control indicated a crucial impact of naturally occurring fungi on ectomycorrhiza formation.

Keywords: container seedlings; ectomycorrhizal inoculation; ectomycorrhiza formation; nursery; seedling morphological quality

Norway spruce (*Picea abies* /L./ Karst) is a widely distributed, ecologically and economically very important coniferous tree species in northern and central Europe (Euforgen 2009; Brus et al. 2012). In central Europe, the stability of Norway spruce (spruce) ecosystems is threatened mainly due to large-scale disturbances caused by windstorms with subsequent bark beetle outbreaks, though drought and heat-induced injuries are more and more frequent as well (Bošeľa et al. 2014; Parobeková et al. 2016; Mezei et al. 2017). Although the incorporation of capable tree species to the stand composition is an essential approach to increase resistance and sustainability of spruce forests, spruce remains a predominating tree species in mountain and subalpine zones (Repáč et al. 2021). Production of a sufficient amount of high-quality spruce seedlings withstanding adverse conditions of planting sites is an important prerequisite to achieve the success of reforestation/afforestation programs.

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In Slovakia, spruce seedlings are predominantly produced as bareroot stock at the age of 3–5 years (1–2 years grown in peat-based substrates and then transplanted to the soil bed) (Repáč, Sendecký 2018). However, outplanting performance of such planting stock type is often rather poor, especially on adverse sites (e.g. large windthrow areas with high-temperature and drought periods, nutrient-poor, water-deficient, polluted or degraded soils, agricultural or other non-forest soils) (Grossnickle, MacDonald 2017). One of the potential tools to increase seedling performance appears to be the use of container stock (Grossnickle, El-Kassaby 2016; Repáč et al. 2021). The advantages of container stock, compared to bareroot stock, are well known (better root protection, ameliorative and nutrient effects of potting substrate, lower shoot to root ratio, greater root growth and drought avoidance potential) (Pinto et al. 2011; Grossnickle, El-Kassaby 2016). Despite that, the proportion of container-grown seedlings in Slovakia is still only about 5% of the total spruce seedling production (15 million annually) (Repáč et al. 2021).

In ectomycorrhizal (ECM) symbiosis, ECM fungi in return for carbohydrates provide several advantages to the ectotrophic host plants (mainly the families *Fagaceae, Betulaceae, Pinaceae* including the genus *Picea*) such as increased water and nutrient uptake, inhibition of plant pathogenic fungi activity, and increased resistance to extremes of environment (Finlay 2008). Since almost all spruce roots in natural forest stands are ectomycorrhizal (Möttönen et al. 2001; Ostonen, Lõhmus 2003), it seems to be feasible that seedlings being outplanted should also be ectomycorrhizal. Seedling roots are mostly naturally colonized with ECM fungi throughout seedling production in a nursery (Rudawska, Leski 2009). Development of seedlings and ectomycorrhizas is considerably affected by physical, chemical and biological properties of growth substrate and cultivation conditions (Brunner, Brodbeck 2001; Repáč et al. 2014; Seyfried et al. 2021). Particularly, usually high water content and fertility of growth substrate increase the seedling aboveground biomass while fine root biomass, root-to-shoot ratio and ectomycorrhiza formation decrease at the same time (Grossnickle, MacDonald 2017; Salcido-Ruiz et al. 2020). Besides, at lower pH and lower substrate fertility, high biomass of ECM taxa with medium or long-distance exploration types is usually present (Seyfried et al. 2021).

Hence, the use of acidic raw humus soils in which mineralization processes are inhibited to the extent that the essential nutrients are present almost exclusively in organic residues and available only to ECM fungi appear to be appropriate for ECM development (Read, Perez-Moreno 2003). In the case of insufficient natural ECM colonization of roots or if specific ECM host-fungi relationships are required, artificial nursery inoculation of the substrate/seedlings with ECM inoculum is needed. Results of experiments concerning the nursery inoculation of spruce seedlings with ECM inocula prepared in laboratory (Le Tacon et al. 1985, 1986; Lehto 1994; Vodnik, Gogala 1994; Repáč 1996; Brunner, Brodbeck 2001; Repáč, Sendecký 2018) showed at least a partial positive effect of inoculation on ectomycorrhiza formation and/or seedling development. Contrary to a small amount of the laboratory-produced inoculum sufficient for small research trials, a large amount of commercially produced ECM inoculum is needed for large-scale operational seedling production.

Several formulations of mycelial and spore inoculum were produced commercially and used on a large scale for the nursery inoculation of millions of bareroot and containerized seedlings substantially increasing reforestation efforts e.g. in the USA (Marx 1991), France (Kropp, Langlois 1990) and later and still in Poland (Sliwa 2009; Sierota 2019). Rossi et al. (2007) and Siddiqui and Kataoka (2011) reviewed some possible techniques of the production of commercial ECM inocula and their availability on the market, and declared the need for encouraging biotechnology research and development in this field to provide maximum benefits to seedling production and outplanting performance. Before large-scale operational use, a small-scale testing of commercial ECM inoculum efficiency considering crucial circumstances affecting ECM and seedling development is necessary. However, just a few trials were carried out to test the nursery performance of commercial inocula in the last decade. Besides the inoculation of *Pinus engelmannii* via injecting a spore solution directly into the growth substrate resulting in inefficiency on seedling survival, growth and ectomycorrhiza formation (Salcido-Ruiz et al. 2020), spruce seedlings were inoculated in other assays. Vuorinen et al. (2015) developed a silica-based propagation medium for the large-scale production of ECM inoculum by solid state fermentation and proved ECM colonization of spruce root systems using this inoculum.

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In Slovakia, commercial mycelial-spore inoculum Ectovit and spore inoculum Mycorrhizaroots were applied into growth substrates in the nurseries to assess their effect on spruce development in juvenile stage (Repáč et al. 2011, 2014; Repáč, Sendecký 2018). The results confirmed a negative impact of fertilizer and pesticide application on the efficacy of the inocula. In consequence, another inoculation trial was recently carried out without chemical application in order to avoid suppression of the root colonization potential of ECM fungi applied in the inocula despite expected weak seedling growth in nutrient-deficient substrate. The objective of this study was to determine the effect of commercial ECM inocula Ectovit and Mycorrhizaroots on the growth and ectomycorrhiza formation of bareroot and container Norway spruce seedlings grown in natural nutrient-poor peat in a forest nursery without additional fertilization and pesticide application. The inoculation treatments were complemented with the application of commercial mineral fertilizer Vetozen.

MATERIAL AND METHODS

Growth substrate and additives. The trial was carried out in a small private forest nursery localized in the north of central Slovakia, Vavrišovo (49°04'N, 19°45'E, altitude 700 m a.s.l., average annual open-air temperature 6.3 °C, average annual precipitation 850 mm). Natural dark high-bog nutrient-poor peat Naturahum (Gramoflor GmbH & CO. KG., Germany) was used as growth substrate. Its low pH ($pH_{(H_2O)}$ = 3.0–5.0), high cation exchange capacity, low inherent fertility and proper balance of aeration and water-holding capacity were expected to provide feasible conditions for ECM development and an adequate seedling growth. The producer specifies the following common properties of the substrate: moisture max. 65%, bulk density max. 200 $g \cdot L^{-1}$, wooden particles (roots, wood chips) max. 3% of weight, dust content max. 20% of weight, burned substances in dry matter min. 85%.

Commercial ECM-hydrogel additive Ectovit (Symbiom s.r.o., Czech Republic), ECM additive Mycorrhizaroots (Lebanon Turf, USA) and natural mineral fertilizer Vetozen (Geoproduct Kft., Hungary) were applied to the growth substrate. The substrate without application of any additive was kept as control. PVC vessels (62 × 40 × 12 cm, length × width × height, sowing area 0.25 m^2) were used as experimental plots in order to prevent cross contamination. Each of the four treatments (three additives + control) was applied to three vessels randomly distributed in three replications (blocks) in open field in the nursery.

Ectovit contained the mycelium of four ECM fungi (*Cenococcum geophilum*, *Hebeloma velutipes*, *Laccaria proxima* and *Paxillus involutus*) and basidiospores of two fungi (*Pisolithus arrhizus* and *Scleroderma citrinum*). The spores were dispersed in a peat-based carrier together with ingredients supporting the development of ectomycorrhizas (humates, ground minerals, extracts from sea organisms) and naturally degradable particles of a water-retaining gel. Ectovit was applied as slurry (gel) that was prepared by mixing the fungal mycelium, the other additive components and adequate amount of water. The slurry was thoroughly mixed with a growing substrate at a ratio 1 : 5 (v : v). The vessels were filled with this mixture immediately before sowing. The number of spores in Ectovit was not known.

Mycorrhizaroots is a powder water-soluble microbial inoculum containing spores of the ECM fungus *Pisolithus tinctorius* (1 600 000 dry $spores \cdot g^{-1}$), four species of *Rhizopogon* (80 000 $spores \cdot g^{-1}$ of each species), two species of *Scleroderma* (40 000) and two species of *Laccaria* (16 000), vesicular arbuscular fungus *Glomus* (8 species) and *Gigaspora margarita* (proportion of all spores is 23.3% of weight), humic acid (28.9%), cold water kelp extracts (18.0%), ascorbic acid (vitamin C) (12.3%), amino acids (glycine) (8.5%), myo-inositol (3.5%), maltodextrin (2.25%), thiamine (vitamin B_1) (2.0%), alpha-tocopherol (vitamin E) (1.0%) and surfactant (0.25%). The substrate in vessels was inoculated with a water solution of Mycorrhizaroots (0.8 $g \cdot L^{-1}$) wetting the root zone thoroughly (approximately 3 $L \cdot m^{-2}$) immediately after sowing and once more 2 weeks after seedling emergence. Irrigation was discontinued for an appropriate time to avoid the runoff of inoculum.

Vetozen is a natural powdery stimulant of seed germination, rooting and growth of cuttings or seedlings providing increased content of macro and micronutrients in the rhizosphere (K, Ca, Mg, Mn, Co, Cu, Fe, Ti, Li). The powdery additive Vetozen was evenly broadcasted with a small sieve on the substrate surface immediately after sowing at the dose of 8 g per vessel (32 $g \cdot m^{-2}$).

Seed sowing and seedling growing. Norway spruce seeds (north Slovakia seed source, localization 49°08'N, 19°46'E, certificate number SK 1070/2010)

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were washed thoroughly for 15 min with tap water, sterilized for 15 min in 30% H_2O_2, then rinsed with distilled water and treated with the fungicide Dithane M-45 (1% of seed weight). Seeds were manually broadcasted (13 g·m^{-2}, approximately 400 seeds per vessel) in the middle of April. The seeds were covered with 0.5 cm layer of the same peat used as growing substrate. The substrate surface was covered with a special textile enabling the soaking of water and providing a mechanical protection to seeds. The textile was raised up to 30 cm above the ground when germination started providing shelter for the seedlings and was removed in a half of the growing season (the end of July). Seedlings were grown under natural environmental conditions.

Before the onset of the third growing season (beginning of April), 35 seedlings from each replicate of each treatment were collected at random and transplanted into plastic trays containing 35 growing cells (PL35F, Lännen Plant Systems – BCC Oy, Säkylä, Finland, each cavity 275 cm^3, depth 130 mm, top 56 × 59 mm, 291 cavities·m^{-2}) filled with the same pure peat substrate as used for the establishment of the trial. The additives were not applied at the time of seedling transplanting. The seedlings were irrigated depending on the rainfall incidence and manually weeded when needed throughout the trial period. Substrate humidity in both vessels and trays varied within the interval of 50–70% of the substrate fresh weight except for periods of substrate overwatering due to rainfall. Neither fertilizers nor pesticides were applied in order to provide a better opportunity for higher efficiency of the additives tested, especially to avoid a potential suppression of the ECM fungi included in the additives due to the inhibitive effect of chemical substances.

Sampling, seedling estimation and data analysis. At the end of each of the three growing seasons (end of October), 15 dormant seedlings per vessel (45 per treatment, 180 in total) were randomly selected and carefully hand lifted. Stem height (*SH*), root collar diameter (*RCD*), and root and shoot dry weights (*RDW* and *SDW*, respectively) (48 h at 80 °C) were recorded for each seedling. Total dry weight (*TDW*) and ratio of root and shoot dry weights (*RDW*/*SDW*) were also calculated. Total number of root tips (*NRT*) of the whole root system was recorded for each 1 + 0 and 2 + 0 seedling. After the third growing season, number of ECM root tips (ectomycorrhizas) and number of all root tips (ectomycorrhizas + non-mycorrhizal roots) were counted on a part of the root system (5–8 randomly selected sections of fine lateral roots, approximately 25 cm of lateral roots in total per seedling) (Rudawska et al. 2006; Repáč et al. 2021) of 8 seedlings of those 15 selected per treatment and replication. For counting of root tips, the root systems were gently washed and observed at 10–25× magnification under a dissecting microscope. The number of root tips per one cm of lateral roots was calculated. ECM morphological types (morphotypes) were distinguished on the basis of gross morphological characteristics of ectomycorrhizas such as ramification, shape, colour, size, outer mantle characteristics, the presence of hyphae and rhizomorphs (Agerer 2012; Ingleby et al. 1990; Agerer, Rambold 2021; Rudawska et al. 2006; Repáč, Sendecký 2018) and described according to the terminology of Agerer and Rambold (2021). The proportion of each ECM morphotype was determined for each estimated seedling as a percentage of the number of ectomycorrhizae of the respective morphotype from the total number of root tips (all ectomycorrhizae + non-mycorrhizal root tips). The percentage of total ECM colonization was determined as a sum of percentages of the morphotypes.

Both bareroot and container experiments were a two-way classification (blocks and treatments) arranged in a randomized complete block design. The experimental unit was the 0.25 m^2 vessel for 1 + 0 and 2 + 0 seedlings and one tray (35 containers) for 2 + 1 seedlings. The growth measurements and total ECM colonization were analysed by one-factorial analysis of variance (ANOVA) followed by Tukey's test (P = 0.05) to determine treatment differences. ANOVA was performed using the SAS statistical package for PC (Version 6.11, 1996).

RESULTS

At the end of the first growing season, no significant differences (P < 0.05) were detected between treatments for any of the estimated growth parameters (Table 1). The applied additives had a significant effect on *RCD*, *SH*, *SDW* and *TDW* of 2 + 0 seedlings. Mean values of these variables in Ectovit-treated seedlings were significantly higher than those in the other treatments including control (Table 1, Figure 1). A similar trend was observed for RDW and NRT but the differences were not significant. One growing season after the transplanting of seedlings to containers (three growing seasons

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Table 1. Analysis of variance (*F* and *P*-values) of the additives effect on the growth parameters of 1-, 2- and 3-year-old, and on the percentage of total ectomycorrhizal (ECM) colonization of 3-year-old Norway spruce seedlings.

Variable	1 + 0 seedlings		2 + 0 seedlings		2 + 1 seedlings	
	F	*P*	*F*	*P*	*F*	*P*
Root collar diameter	0.91	0.4883	5.09	0.0435	7.10	0.0212
Stem height	0.55	0.6669	12.13	0.0059	9.52	0.0107
Shoot dry weight	1.26	0.3682	7.56	0.0184	23.52	0.0010
Root dry weight	1.46	0.3157	3.88	0.0743	30.15	0.0005
Total dry weight	1.29	0.3605	6.67	0.0240	27.12	0.0007
Root-to-shoot ratio	1.99	0.2019	2.45	0.1614	3.06	0.9475
Number of root tips	2.01	0.2145	1.61	0.2834	0.85	0.5161
Total ECM colonization	–	–	–	–	0.40	0.7693

Degrees of freedom for all parameters of 1 + 0 and 2 + 0, and for growth parameters of 2 + 1 seedlings: error *df* = 6; residual *df* = 168; total *df* = 179; degrees of freedom for root tips and ECM colonization of 2+1 seedlings: residual *df* = 84; total *df* = 95

Table 2. Description of ectomycorrhizal (ECM) morphological types of 2 + 1 container Norway spruce seedlings grown in peat substrate inoculated at sowing with the commercial ectomycorrhizal additives Ectovit and Mycorrhizaroots and mineral rooting stimulator Vetozen

ECM morphotype	Description	Photo (15× maginification)
Dark brown thin	monopodial-pinnate ramification, ramification order 0–1, tortuous, different lengths, mostly beaded, smooth and dull mantle surface, brown colour, brown tips, ochre hyphae	
Brown	monopodial-pinnate ramification, ramification order 0–1, straight, various length, thickened toward the root tips, shiny smooth mantle surface, dark brown colour, ochre tips, no emanating hyphae	
Greyish-brown	monopodial-pyramidal ramification, ramification order 0–1, cylindrical – constricted at the point of ramification, slightly bent, various length, shiny smooth mantle surface, greyish colour, whitish tips, whitish hyphae	
Dark brown thick	monopodial-pyramidal ramification, ramification order 0–2, numerous clumps of different arrangement and lengths, tapering toward root tips, smooth shiny mantle, brown to black colour, yellow tips, whitish hyphae	
Paxillus-like	monopodial-pinnate or pyramidal ramification, ramification order 0–1, variously shaped (strait to tortuous), chaotically arranged (solitary to dense), slightly swollen toward root tips, dull grainy mantle surface, brown, whitish tips, downy white mycelium and densely white hyphae	

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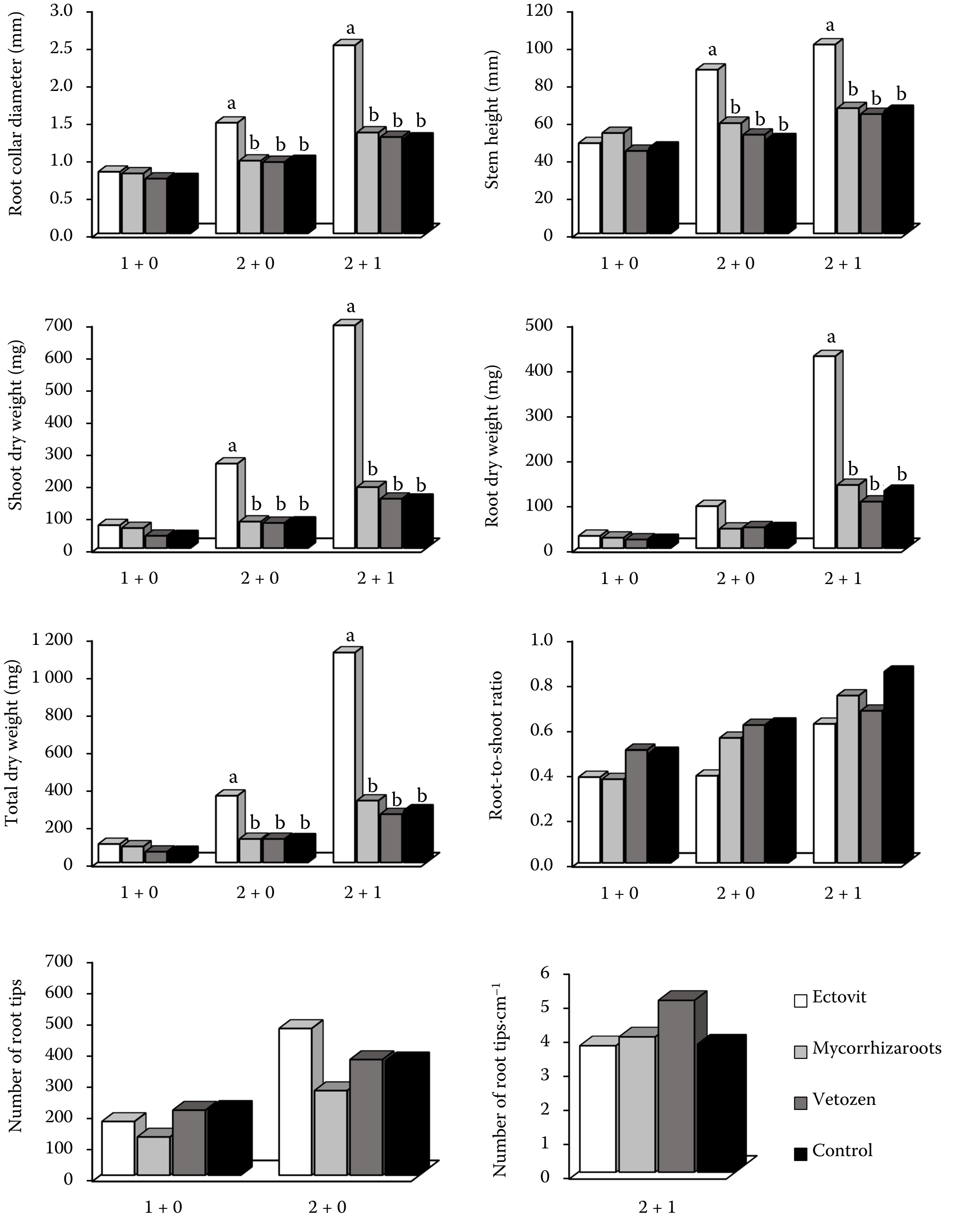

Figure 1. Mean values of growth parameters of 1-, 2-, and 3-year-old *Picea abies* seedlings grown in peat substrate with the application of commercial additives.

Within a seedling age value without a letter or followed by the same letter are not significantly different (Tukey test, $P > 0.05$)

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Table 3. Mean values of relative abundance of ectomycorrhizal morphotypes and total ectomycorrhizal colonization (± standard deviation) of 2 + 1 container Norway spruce seedlings grown in peat substrate treated with commercial additives at sowing [significance of differences among treatments was tested only for total ectomycorrhizal colonization; a significant difference was not found (Tukey test, $P > 0.05$)]

Additives	Ectomycorrhizal morphotypes (%)					Total colonization ± SD
	brown	dark brown thin	dark brown thick	greyish-brown	*Paxillus*-like	
Ectovit	22.3	47.6	0.8	0.0	7.8	78.4 ± 24.3
Mycorrhizaroots	34.8	39.1	1.7	0.0	0.0	75.5 ± 21.4
Vetozen	33.4	30.5	0.0	2.2	0.0	66.2 ± 27.2
Control	19.2	59.4	0.0	2.0	0.0	80.5 ± 21.6

after sowing and inoculation), the differences between Ectovit and the other treatments increased, with the exception of *NRT* and *RDW*/*SDW*. Growth differences between Mycorrhizaroots, Vetozen and control treatments were very low (Figure 1). The mean values of *TDW*, *SDW*, *RDW*, *RCD* and *SH* of the Ectovit-inoculated seedlings were higher by about 300%, 290%, 260%, 90% and 50%, respectively, than those in the other treatments including control. *NRT* in Ectovit and Vetozen treatments were higher than in the other treatments after the second and third year, respectively, but the differences were not significant. *RDW*/*SDW* was higher after the third year than after previous years in all treatments and was slightly lower in Ectovit than in the other treatments (Figure 1).

Our effort to reliably identify ectomycorrhizas of 2 + 1 seedlings on a species or genus level according to gross morphological features was not unequivocally successful, so the ectomycorrhizas were typed to particular experimental ECM morphotypes. The morphotypes were described according to Agerer and Rambold (2021) terminology (Table 2).

Seedling roots in all treatments were quite well colonized by ECM fungi. Total ECM colonization was 66.2–80.5% depending on the treatment. The highest colonization was recorded in control and the lowest in Vetozen, but no significant difference between these treatments was found ($P = 0.769$) (Table 3). However, pronounced differences in relative abundance of the morphotypes occurred both between and within treatments. Non-mycorrhizal roots of seedlings in all treatments were thin, translucent, lacking a mantle and with root hairs.

A treatment-specific *Paxillus*-like morphotype was found in Ectovit treatment; this morphotype accounted for about one tenth of ectomycorrhizas in the Ectovit-treated seedlings and did not occur in the other treatments. Dark brown thin ectomycorrhizas were the most abundant; they reached rather over a half amount of all ectomycorrhizas within each (except for Vetozen) treatment (30.5–59.4% of all root tips depending on the treatment) (Table 3). The Mycorrhizaroots- and Vetozen-treated seedlings had an almost 2-fold higher proportion of brown ectomycorrhizas than those from the Ectovit and control treatments. The occurrence of dark brown thick and greyish-brown morphotypes was very rare, whereby the former were detected on both Ectovit- and Mycorrhizaroots-inoculated seedling, and the latter on both Vetozen-treated and control seedlings (Table 3).

DISCUSSION

Outplanting performance of seedlings under a particular set of site conditions often depends on considerations and decisions made prior to planting including stock type choice and quality of seedlings used (Rose et al. 1991; Grossnickle, MacDonald 2017). Morphological attributes are the most common measures relating seedling quality to field performance (Pinto et al. 2011). Larger-size seedlings achieved better performance than the smaller-size ones in various planting site environmental conditions (Ivetić et al. 2016; Repáč et al. 2021), especially on sites with intensive vegetative competition (Hytönen, Jylhä 2008; Johansson et al. 2015; Gallo et al. 2020). However, smaller-size container seedlings of various tree species proved better survival over larger-size bareroot seedlings, especially on adverse sites (Renou-Wilson et al. 2008; Grossnickle, El-Kassaby 2016). This finding could potentially alleviate a disadvantage of the smaller size of the container seedlings cultivated in our trial (due to the absence of fertilizer application) compared to the size of operationally produced fertilized seedlings and to the size

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recommended by the respective national standard. Shorter seedlings with an adequate root system can have an advantage on water stressed sites (Jurásek et al. 2009; Grossnickle, El-Kassaby 2016) because the root systems of taller seedlings being exposed to water stress cannot supply enough water to transpiring foliage to maintain a proper water balance (Grossnickle 2012).

Regarding individual growth attributes, the initial root collar diameter, a very easily measured morphological attribute, appears to be the best morphological attribute to forecast future growth (Omi 1991) because it often correlates with seedling weight and root system size (Mexal, South 1991; Grossnickle 2012). The root system is considered important because the greater root system size provides a greater root absorptive surface for water uptake (Grossnickle 2012). Appropriate proportionality between the shoot and the root system (shoot-to-root ratio) is a desirable plant attribute conferring a greater potential of seedling drought avoidance (Grossnickle, El-Kassaby 2016). In our trial, *RDW*/*SDW* increased in all treatments (by 46% regardless of the treatment) after transplanting seedlings to the containers (2 + 1) compared to that of 2 + 0 seedlings. This result is the most probably due to a larger space provided in the containers for the roots intensively searching nutrients in the nutrient-deficient pot substrate. Although *RDW*/*SDW* of the Ectovit-inoculated 2 + 1 seedlings was slightly lower than that in the other treatments, the ratio of Ectovit-treated 2 + 1 seedlings compared to Ectovit-treated 2 + 0 seedlings increased more than the ratio compared between container and bareroot seedlings in the other treatments. It is so because the root biomass increased more times than the aboveground biomass of 2 + 1 container against 2 + 0 bareroot seedlings in Ectovit in comparison with the other treatments. Equally, *RCD* annual increment of Ectovit-inoculated container seedlings was higher than that of the seedlings in the other treatments. Thus, a significantly promotive effect of Ectovit on the growth attributes having a high potential to forecast future seedling growth should provide a potential advantage facilitating the better performance of Ectovit-inoculated outplanted seedlings.

No stimulative effect of Ectovit in the growing season following its pre-sowing application unlike the increasing positive effect in the next two years indicates a delayed and longer-term impact of the inoculum on the seedling growth under trial conditions. As 2 + 0 seedlings grew continually in the inoculated substrate, favourable effects of the hydrogel, ingredients and/or ECM fungi contained in Ectovit on the seedling growth were conferred the most probably via improvement of physical, nutritional and microbial properties of the Ectovit-inoculated substrate. Higher increments of the parameters of 2 + 1 seedlings transplanted from Ectovit-inoculated substrate to pure peat pot substrate compared to those from the other treatments were the most probably facilitated by possible profits of seedlings gained from Ectovit-inoculated substrate, such as larger size, higher nutrient content in tissues, better physiological quality, and beneficial microbial community on the roots including ECM fungi.

In the previous studies, effects of Mycorrhizaroots and Vetozen on the growth and ECM colonization of spruce seedlings (Repáč et al. 2014) and cuttings (Repáč et al. 2011) were insignificant, similarly like in this study. Likewise, in small nursery inoculation trials no significant effect of Ectovit on spruce development was recorded in most treatments (Repáč et al. 2011, 2014; Repáč, Sendecký 2018). The fertilizer as a component of the growth substrate composition and/or the application of fungicides and fertilizers during the growing period likely had a negative effect on the applied ECM fungi (Smaill, Walbert 2013). Nevertheless, inoculation with Ectovit stimulated seedling growth and increased needle P and K concentrations in nutrient-not-enriched peat substrate contrary to nutrient-enriched one despite subsequent fertilization during seedling production (Repáč et al. 2014). A positive influence of Ectovit on the needle nutrient concentration of spruce cuttings was also described by Repáč et al. (2011). However, the above-mentioned authors concluded that the observed positive effect of Ectovit was probably caused by its physical and chemical properties rather than by the efficiency of applied ECM fungi, because the used fungi were not traced in the ectomycorrhizas by DNA analysis and did not form a specific Ectovit treatment-related ECM morphotype. But nutritional or non-nutritional effects of the applied fungi on the seedling development were not fully excluded.

Although the seedling growth promotion is a desired benefit, it is not a crucial goal of the nursery ECM inoculation. The inoculation goals are

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primarily induction and promotion of ectomycorrhiza formation as it is an important contributor to the survival potential of seedlings after outplanting to forest sites (Sanchez-Zabala et al. 2013) and biological control of pathogens (Tahat et al. 2010). Unfortunately, the extensive ECM colonization in all treatments including the control and relative abundance of the ECM morphotypes in this trial indicated low effectivity of the applied fungi and dominant impact of naturally occurring indigenous fungi in the formation of ectomycorrhizas probably originating from the peat used or from natural air-borne propagules. Ectomycorrhiza formation by indigenous nursery fungi despite artificial inoculation was also reported e.g. by Ingleby et al. (1994), Sanchez-Zabala et al. (2013), Trakal et al. (2013) and Vuorinen et al. (2015). Rudawska et al. (2006) and Pietras et al. (2013) found that naturally occurring ECM fungal communities in forest nurseries are quite diverse and the level of seedling colonization is usually high. High total ECM colonization with indigenous fungi but relatively poor diversity of ECM morphotypes of spruce seedlings were reported by Repáč et al. (2014) and Repáč and Sendecký (2018). Maltz and Treseder (2015) declared that ECM fungi applied in inocula might be less effective mutualists than naturally occurring ECM fungi yielding higher ECM colonization due to environmental conditions more familiar to indigenous than introduced ECM fungi.

It is impossible to say which component(s) contained in Ectovit had a crucial positive influence on the seedling growth in our study. Any effects of composite hydrogel and chemical ingredients (Heiskanen 1995; Chirino at al. 2011; Repáč et al. 2014) are very feasible. Nevertheless, as the effect of Ectovit was apparent only since the second year after its application, it seems that one of the circumstances influencing seedling growth may be the development of ECM community. More importantly, the occurrence of *Paxillus*-like morphotype explicitly in Ectovit treatment encourages us to presume that this morphotype was the most probably formed by *Paxillus involutus* involved in Ectovit. Read and Perez-Moreno (2003) and Korkama et al. (2006) proved that the ECM community with *P. involutus* provides good nutrient uptake of seedlings resulting in the good tree growth rate. Kwaśna and Szewczyk (2016) observed that seedlings inoculated with some fungi developed more foliage than seedlings inoculated with other ones resulting in faster seedling development and higher biomass production. Moreover, non-nutritional effects of inoculation (e.g. growth hormones produced by fungi, impact on the spectrum of soil microorganisms, protection against environmental stress) may influence seedling development (Ingleby et al. 1994; Trakal et al. 2013). However, because the identification of root- and substrate-associated ECM fungi was not done by molecular methods, the participation of the applied fungi in ectomycorrhiza formation and seedling growth stimulation is not verified. Any of these above-mentioned effects of inoculation might be considered to potentially provide the stimulating effect of the ECM fungi (especially *P. involutus*) contained in Ectovit on the seedling growth in our trial. The efficacy of inoculation may be affected by various circumstances (mycelium viability, inoculum type, inoculation technique, symbiotic partner compatibility, seedling production environment and others) and may vary from positive (Brunner, Brodbeck 2001; Repáč 1996; Sanchez-Zabala et al. 2013) through insignificant (Aleksandrowicz-Trzcińska et al. 2013; Repáč et al. 2014; Repáč, Sendecký 2018) to even reduced seedling growth (Eltrop, Marchner 1996; Rincón et al. 2001; Kwaśna, Szewczyk 2016) caused by the depletion of seedling assimilates by introduced symbiotic fungi.

CONCLUSION

Estimation of the effect of commercial ECM inocula Mycorrhizaroots and Ectovit, and of mineral fertilizer Vetozen on the growth and ectomycorrhiza formation of Norway spruce seedlings revealed that Ectovit was the only efficient additive among those tested in terms of seedling growth improvement and formation of treatment-specific ECM root morphotype. It is impossible to determine which component(s) of multi-compound composite Ectovit and in which range influenced this effect. Although the presence of treatment-specific ECM morphotype in Ectovit treatment suggests the stimulative effect of the applied fungi on seedling growth, the extensive occurrence of the other ECM morphotypes in all treatments including the control indicates decisive participation of naturally occurring ECM fungi in ectomycorrhiza formation. Due to initial low substrate fertility and absence of fertilization and pesticide application, the root-to-shoot ratio and ECM colonization of roots were appro-

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priate but the seedling size was too small. Thus besides testing of various circumstances of Ectovit application and conditions of seedlings production, the definition of an adequate fertilization level that does not suppress the positive effects of ECM fungi applied and at the same time promotes the growth of seedlings should be useful in future research.

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